Patterning of the vertebrate embryo along the left–right (L/R) axis is required for proper positioning and asymmetric development of the visceral organs. The conserved role of the Nodal signaling pathway during this process has been well established, but how asymmetric gene expression is interpreted by tissues to result in asymmetric morphogenesis is still not well understood. To address this question, we have studied the processes of cardiac jogging and looping in zebrafish. We find that Nodal signaling influences the direction of myocardial migration within the cardiac cone, just prior to jogging, and that the direction of these cellular movements are reversed in mutants with defects in asymmetric gene expression. In addition, we find that this event results in a repositioning of the original L/R axis to the dorsal–ventral (D/V) axis of the linear heart tube. Finally, we have discovered the existence of a rotation within the heart tube just prior to cardiac looping which converts the D/V axis back to the L/R axis. While the direction of this rotation is reversed in morphants with defects in asymmetric gene expression, the reestablishment of the original L/R axis occurs properly, regardless of Nodal signaling laterality. These results suggest a role for asymmetric gene expression in directing the first axis conversion during cardiac jogging but indicate that the second axis conversion at the initiation of cardiac looping may occur in a Nodal-independent manner.

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Program/Abstract # 458

3-*O*-sulfotransferase is required for cardiac development and physiology in zebrafish

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Heart development involves precise coordination of patterning events, cell movements and cell physiology in order to generate a functional heart. Cell surface and extracellular heparan sulfate proteoglycans (HSPGs) are core proteins with modified glycosaminoglycan (GAG) chains that are thought to mediate interactions between cells and their environments. We have cloned multiple 3-*O*-sulfotransferases (3-OSTs)1 presumed to add sulfate to the 3-position carbon of GAGs in HS, and are systematically assessing their roles in development. Morpholino knockdown of one of the 3-OST family members, 3-OST-7, results in a hypoplastic cardiac ventricle that does not contract properly, resulting in poor blood circulation and pericardial edema. What is the underlying mechanism for the observed cardiac ventricular defect? Primary heart field specification and patterning, and development of the vasculature and atrioventricular valve appear to be normal. In contrast, the outflow tract fails to form properly in morphant embryos. Moreover, action potential and intracellular calcium measurements indicate that ventricular contraction is uncoupled from excitation. Together these results indicate that 3-OST-7 has multiple roles in heart development, and that 3-OST function might provide a novel mechanism for the regulation of cardiac cell physiology. 1. Cadwallader AB, Yost HJ. Combinatorial expression patterns of heparan sulfate sulfotransferases in zebrafish: I. The 3-*O*-sulfotransferase family. Dev Dyn. 2006;235:3423-31.

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Program/Abstract # 459

Channel independent functions of L-type calcium channel beta-2 subunit

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Calcium channel beta-2 (CACNB2) subunits regulate voltage-gated channel electrophysiological dynamics and chaperon newly synthesized pore-forming alpha subunits to the plasma membrane. In addition to these canonical roles as calcium channel modulators, recent studies indicate that the beta subunits may have channel-independent functions that relate to their MAGUK (Membrane Associated Guanylate Kinase) protein structure. MAGUK family proteins perform a variety of scaffolding functions in the cell. We report the discovery of two CACNB2 genes in zebrafish. We find that the cardiac cells of CACNB2 morpholino-treated embryos dissociate more easily under pressure, and are reduced in number. To determine if cardiac myocyte morphology and cell adhesion is compromised in beta-2 morphants, we assayed cardiac cellular organization with several membrane markers including N-Cadherin.

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Program/Abstract # 460

Tbx5-mediated β2 CaMK-II expression is required for heart looping and pectoral fin development

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Mutations in the gene encoding Tbx5, result in Holt–Oram syndrome (HOS), which is characterized by defective cardiac development and stunted forelimbs. The genetic targets of Tbx5 responsible for proper heart and limb development are still being identified. In zebrafish embryos, the functional suppression of type II Ca^{b+}/calmodulin dependent protein kinase (CaMK-II) results in both aberrant cardiac looping and diminished pectoral fin development similar to tbx5 morphant and mutant (*heartstrings: hst*) embryos. Morphants of just one of the seven genes encoding catalytically active CaMK-II in early zebrafish embryos (β2 CaMK-II; *camk2b2*) exhibit the *hst* phenotype. *Camk2b2* mRNAs are transiently expressed in the heart and limb buds at the time of heart looping. Cardiac abnormalities in *camk2b2* and *tbx5* morphants can be reversed by overexpression of cytosolic CaMK-II. Normal fin development can be restored by CaMK-II in *camk2b2* morphants, but not in *tbx5* morphants. Both *tbx5* morphant and *hst* embryos exhibit diminished β2 CaMK-II, while the introduction of excess Tbx5 into zebrafish embryos and mouse fibroblasts increases β CaMK-II expression. Tbx5 also promotes transcription of a *camk2b2*-reporter, most likely through direct interaction with the evolutionarily conserved Tbx5 binding elements found in β CaMK-II genes. These findings indicate that Tbx5 induces β CaMK-II expression, which is necessary for normal cardiac and limb development.

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Program/Abstract # 461

Hedgehog signaling plays a cell-autonomous role in maximizing cardiac developmental potential

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Elucidation of the complete roster of signals required for cardiac specification is critical to the future of regenerative medicine. Prior studies have implicated the Hedgehog (Hh) signaling pathway in the regulation of multiple aspects of heart development. However, our understanding of the contribution of Hh signaling to the initial specification of myocardial progenitor cells remains incomplete. Here, we show that Hh signaling promotes cardiomyocyte formation in zebrafish. Reduced Hh signaling creates a cardiomyocyte deficit, and increased Hh signaling creates a cardiomyocyte surplus. Through fate mapping, we find that Hh signaling is required at early stages to insure specification of the proper number of myocardial progenitors. Genetic inducible fate mapping in mouse indicates that myocardial progenitors respond directly to Hh signals, and transplantation experiments in zebrafish demonstrate that Hh signaling acts cell autonomously to promote contribution to the myocardium. Thus, Hedgehog signaling plays an essential early role in maximizing cardiac developmental potential, making it an attractive target for manipulation of multipotent progenitor cells.

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Program/Abstract # 462
Expression patterns of *sox9* gene during chick heart development
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SOX gene family encodes a set of transcription factors that are involved in sex determination and various developmental processes. We cloned *sox9* gene from chick embryonic heart, and examined their expression patterns in the developing heart from stage 14 to 33 by in situ hybridization. In the looped heart at stages14, *sox9* mRNA was detected in the myocardium of outflow tract (OT). At stage 16, *sox9* was expressed in the activated-endothelial cells of the OT and atrioventricular (AV) canal regions, in which valvuloseptal endocardial cushion tissue would later develop. Thereafter *sox9* was expressed in the endothelial and mesenchyma cells in cushion tissue until stage 33, but not in the myocardium. In addition, *sox9* mRNA was detected in the various tissues, such as the neural tube, notochord, somite, limb, mesonephros, primordial gut, lung bud and developing bone. Results suggest that *sox9* may play an important role in migration and invasion of the endothelial cells during epithelial–mesenchymal transition in cushion tissue formation and cell differentiation in other organogenesis during chicken development.

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Program/Abstract # 463
Endothelial deletion of PlexinD1 results in congenital heart, vascular and skeletal defects
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PlexinD1 is a membrane bound receptor that is thought to function by mediating signals derived from class 3 secreted semaphorins. Although the role of semaphorin signaling in axon guidance in the nervous system has been extensively studied, functions outside the nervous system including important roles in vascular patterning have also been demonstrated. Inactivation of PlexinD1 leads to neo-natal lethality, structural defects of the cardiac outflow tract and peripheral vascular abnormalities. PlexinD1 is expressed by vascular endothelial cells, but additional domains of expression have also been demonstrated including lymphocytes, osteoblasts and the central nervous system. Hence, the cell-type specific functions of PlexinD1 have remained unclear. Here, we describe the results of tissue-specific gene inactivation of PlexinD1 in Tie2 expressing precursors, which recapitulates the null phenotype with respect to congenital heart, vascular and skeletal abnormalities. In addition, we demonstrate functions for PlexinD1 in post-natal retinal vasculogenesis through the use of inducible cre-mediated deletion.

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Program/Abstract # 464
Snail1 transcription factor in bone development and homeostasis
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The Snail gene family of transcription factors is fundamental during embryonic development in processes that imply cell movements including mesoderm and neural crest formation. However, they must be maintained silent in the adult as their pathological activation leads to several pathologies. As such, its aberrant activation in tumours leads to the acquisition of invasive and metastatic properties while its activation in the adult kidney leads to renal fibrosis. Both involve the Snail-mediated induction of the EMT. In addition, Snail factors attenuate cell proliferation and induce resistance to cell death, necessary for normal embryonic and malignant tumour cells to form organs or metastasis, respectively. Interestingly, Snail also functions in non-epithelial cells, such as chondrocytes, where it is unable to induce EMT but still controls proliferation. Indeed, its deregulated expression in the developing bone leads to achondroplasia in transgenic mice, the most common form of dwarfism in humans. Achondroplasias are associated with activating mutations in FGFR3. Snail1 is the transcriptional effector of FGFR3 signaling as the inhibition of Snail1 abolishes its signaling even through the pathological activating FGFR3 forms. Snail1 expression is very tightly regulated in the bone and after having shown its importance during fetal bone development we wondered whether its aberrant activation in the adult had any impact on bone homeostasis. Our preliminary data indicate that indeed, Snail1 activation disrupts mineralization in the adult bone.

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Program/Abstract # 465
Making and shaping seamless tubes
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Although tubular networks form organs of strikingly different shapes, the tubes that compose them obey remarkably similar architectural design principles. Hence, understanding the logic underpinning tube formation in one organ system is likely to be highly relevant to other organ systems as well. Subcellular tubes such as the